

I Claim:

1. A method for amplifying the presence of an actively respiring microorganisms in a sample comprising contacting the contents of said sample to a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms produces a viability marker.
2. The method of claim 1 wherein the microorganisms comprise bacteria.
3. The method of claim 1 wherein the viability substrate is triphenyltetrazolium, nitrotetrazolium blue, iodonitrotetrazolium or dimethylthiazolyldiphenyl tetrazolium.
4. The method of claim 1 wherein the nutrient media contains glucose and NADH.
5. The method of claim 1 wherein the viability marker is a water insoluble marker that accumulates in direct proportion to the number of microorganisms of said sample.
6. A method for detecting an actively respiring microorganisms in a sample comprising:
 - trapping the microorganisms on a solid filtration membrane;
 - treating the microorganisms according to the method of claim 1;
 - digesting the microorganisms;
 - contacting primary antibodies prepared against a substituted formazan with the digested microorganisms to capture said primary antibodies;
 - contacting secondary antibodies prepared against the primary antibodies and conjugated with a detectable marker to captured primary antibodies; and
 - detecting the secondary antibodies that are bound to the captured primary antibodies.
7. A method for detecting microorganisms whose presence is amplified by the method of claim 1 comprising:
 - digesting the microorganisms by incubation with a lysozyme to form a cellular debris, wherein the viability marker is adsorbed on a surface of the cellular debris;
 - immobilizing primary antibodies specific for the viability marker on a solid support;

contacting the digested microorganisms with the immobilized primary antibodies thereby capturing the microorganisms; and

detecting the presence of the viability marker.

8. The method of claim 7 wherein the step of detecting comprises:

5 contacting the captured digested microorganisms with a reporter antibody prepared from the primary antibody, the reporter antibody being conjugated to a detectable marker; and

 detecting the reporter antibodies that bind to the captured digested microorganisms.

10 9. The method of claim 7 wherein the step of detecting comprises detecting the captured viability marker by detecting a change in a physical, a chemical, an optical, or an electrical property of the solid support.

10. The method of claim 7 further comprising the steps of:

15 incubating the viability marker with a primary antibody specific for the viability marker and conjugated to a reporter molecule, thereby forming a primary antibody-antigen-reporter molecule sandwich; and

 detecting the reporter molecule.

11. A method for detecting microorganisms according to the method of claim 1 comprising:

20 digesting the microorganisms;

 incubating the digested microorganisms with a primary antibody specific for the viability marker;

 conjugating the primary antibody to a reporter molecule to form a reporter-primary antibody complex; and

25 detecting the reporter molecule.

12. A method for detecting an actively respiring microorganisms in a sample comprising:

 treating the microorganisms according to the method of claim 1;

 digesting the microorganisms;

contacting a primary antibody prepared against a substituted formazan with the digested microorganisms;

contacting a secondary antibody prepared against the primary antibody, the secondary antibody being conjugated to a reporter molecule; and

5 detecting the reporter molecule.

13. The method of claim 12 further comprising the step of trapping the actively respiring microorganisms on a solid filtration membrane.

14. The method of claim 12 wherein the reporter molecule comprises an enzyme, a bioluminescent protein, a radioisotope, a chemiluminescent dye, a visible dye, a latex
10 particle, a magnetic particle or a fluorescent dye.

15. The method of claim 12 wherein the sample is a clinical sample, a food sample, a cosmetic sample, a pharmaceutical sample, an industrial sample or an environmental sample.

16. The method of claim 12 wherein the sample is a blood sample, a tissue sample,
15 a tissue homogenate sample or a bodily fluid sample.

17. The method of claim 12 wherein the microorganisms comprises a single species of microorganisms or a mixed population of microorganisms.

18. The method of claim 12 wherein the sample contains less than 1000 cfu/mL.

19. The method of claim 12 wherein the detecting takes less than two hours.

20. Monoclonal or polyclonal antibodies prepared to a substituted formazan and cross reactive to other formazans.

21. A kit for the rapid and sensitive detection of viable microorganisms comprising:
means for amplifying the presence of a microorganisms in a sample; and
means for detecting the microorganisms.

25 22. A method for diagnosing a disease due to a microorganisms comprising:
amplifying the presence of the microorganisms by the method of claim 1;
digesting the microorganisms;

contacting a primary antibody prepared against a substituted formazan with the digested microorganisms;

contacting a secondary antibody prepared against the primary antibody, the secondary antibody being conjugated to a reporter molecule; and

5 ✓ detecting the reporter molecule.

23. A method for quantitating actively respiring microorganisms in a sample comprising:

 contacting said microorganisms to a nutrient medium containing a predetermined amount of a tetrazolium salt;

10 metabolizing the tetrazolium salt to a viability marker using the microorganisms;

 forming a quantitative amount of the viability marker that reflects the quantity of actively respiring microorganisms in the sample; and

 ✓ detecting the viability marker.

15 24. A method for viability-marking an actively respiring microorganisms in a sample comprising contacting the contents of said sample to a nutrient medium containing a predetermined amount of a viability substrate, wherein metabolism of said viability substrate by the microorganisms of said sample produces a viability marker.

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